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## HUMAN RECOMBINANT ERYTHROPOIETIN GRADIENT DOSAGE INFLUENCE ON ISCHEMIC AND REPERFUSION LIVER INJURY

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**Abstract.** We investigate influence of human recombinant erythropoietin different dosage on blood flow velocity and morphological changes in liver during ischemic and reperfusion injury. It was proved what optimal preconditioning dosage is 50 IU/kg.

**Key words:** ischemia, reperfusion, liver, blood flow, morphological changes.

Ischemic and reperfusion complications take leading role in development internal organs different diseases as liver injury during surgical interventions with decreasing blood supply.

The possibility of direct and remote ischemic preconditioning simulation is an experimental pharmacology keystone.

The greatest interest among a number of substances for pharm modeling ischemic preconditioning, it is a recombinant human erythropoietin showed to be effective in protecting to myocardial and brain tissues ischemia/reperfusion injuries protection [4, 5].

However data of human recombinant erythropoietin usage in liver ischemia/reperfusion correction are rather contradictory.

The goal of our investigation was to assess the influence of human recombinant erythropoietin different dosage on blood flow velocity as microcirculation changes evaluation screening method.

Materials and methods: 70 white (male and female) 280-300g rates were used. Animals were grouped in 7 sets by 10 rates each. I/R group: reperfusion 30 minutes followed by 30 minutes of ischemia. I/R+EPO in dose 5 IU/kg group: reperfusion 30 minutes followed by 30 minutes of ischemia pretreated with 5 IU/kg human recombinant erythropoietin. I/R+EPO in dose 25 IU/kg group: reperfusion 30 minutes followed by 30 minutes of ischemia pretreated with 25 IU/kg human recombinant erythropoietin. I/R+EPO in dose 50 IU/kg group: reperfusion 30 minutes followed by 30 minutes of ischemia pretreated with 50 IU/kg human recombinant erythropoietin. I/R+EPO in dose 100 IU/kg group: reperfusion 30 minutes followed by 30 minutes of ischemia pretreated with 100 IU/kg human

recombinant erythropoietin. I/R+EPO in dose 200 IU/kg group: reperfusion 30 minutes followed by 30 minutes of ischemia pretreated with 200 IU/kg human recombinant erythropoietin. I/R+EPO in dose 500 IU/kg group: reperfusion 30 minutes followed by 30 minutes of ischemia pretreated with 500 IU/kg human recombinant erythropoietin. All interventions were made under general anesthesia («Zolitel 100» 60 mg/kg with chloral hydrate 125 mg/kg intraperitoneally).

Transient deep liver ischemia reproduced by temporary hepatoduodenal ligament compression for 30 min [1, 2, 3].

Human recombinant erythropoietin («Epocrin» obtained from StateSRU ultrapure biological drugs FMBA FGUP, Russia) injected intraperitoneally 50 IU/kg 30 min before ischemia.

Blood flow velocity was measured by Biopaq systems MP150 with TSD144 probe in perfusion units (PU).

Direct ischemic preconditioning was reproduced 30 min ahead of deep ischemia episode by 10 min hepatoduodenal ligament compression.

For control method we used standard histological investigation with hematoxylin/eosin dye.

During research we found blood flow velocity were on  $850.48 \pm 19.75$  PU level. Deep ischemia episode leads to perfusion dropping to zero level with restoration on 1 minute till  $120.17 \pm 4.7$  PU, changed with transient hyperemia  $1983.22 \pm 63.35$  PU on 15 minute and decreasing till  $611.63 \pm 27.43$  PU on reperfusion 30 minute. According obtained data the best time for assessing is reperfusion 15 minute as maximum volatile point.

Direct ischemic preconditioning largely decrease transient hyperemia till  $1338.46 \pm 14.06$

PU on 15 minute, changed by  $500.16 \pm 16.41$  PU on 30 minute blood flow velocity investigation.

Human recombinant erythropoietin injection in doses 5, 25, 50, 100, 200, 500 IU leads to transient hyperemia decreasing with maximum effect in 200 and 500 IU/kg doses (Table 1).

Statistic analysis revealed no differences between groups with 200 and 500 IU/kg, moreover small distinctions between groups with 50 and 100 IU/kg were found and dose of 50 IU/kg we decided to use later as safer one.

Table 1

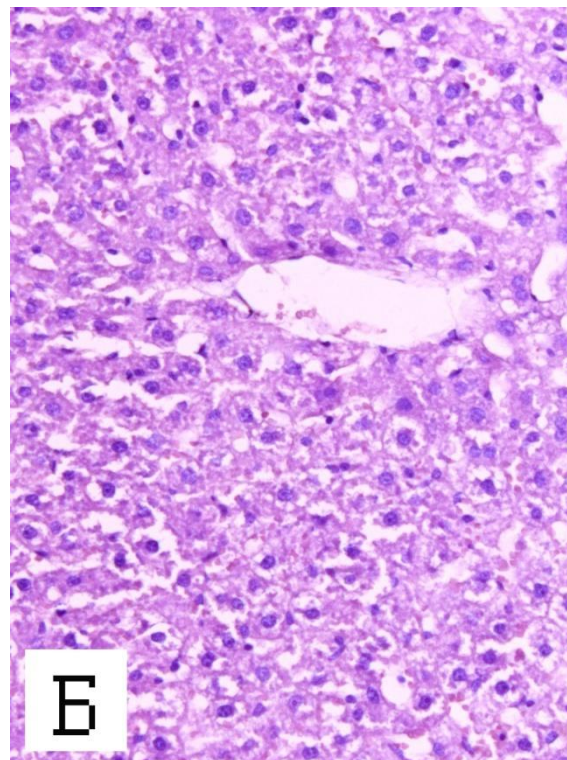
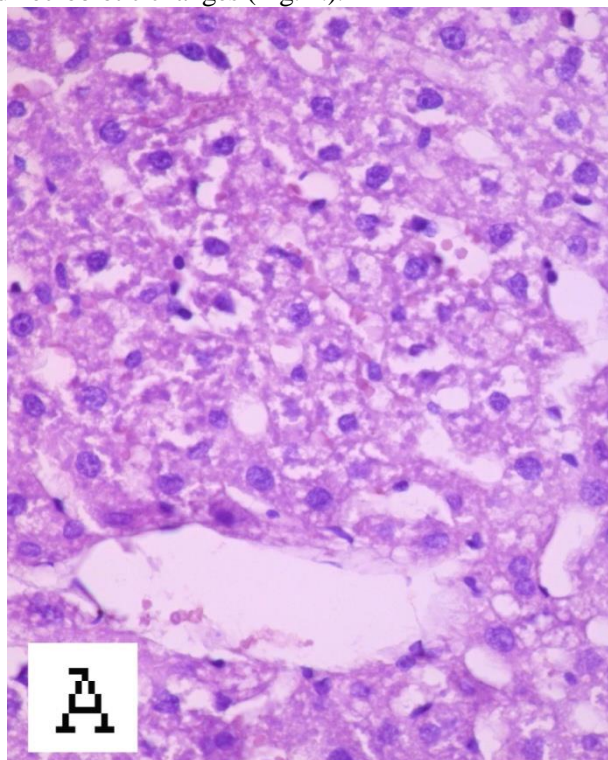
**Human recombinant erythropoietin different dosage effect on blood flow velocity in liver microvascular vessels during ischemia and reperfusion (PU) ( $M \pm m$ ,  $n=10$ )**

Groups	15 reperfusion minute
I/R	$1983.22 \pm 63.35^*$
I/R+EPO in dose 5 IU/kg	$1856.38 \pm 72.12^{**}$
I/R+EPO in dose 25 IU/kg	$1789.28 \pm 22.58^{**}$
I/R+EPO in dose 50 IU/kg	$1447.93 \pm 23.72^{**}$
I/R+EPO in dose 100 IU/kg	$1367.81 \pm 34.28^{**}$
I/R+EPO in dose 200 IU/kg	$1295.26 \pm 54.82^{**}$
I/R+EPO in dose 500 IU/kg	$1308.14 \pm 31.87^{**}$
I/R+IPC	$1338.46 \pm 14.06^{**}$

Note \* –  $p \leq 0.05$  versus against intact group data, \*\* –  $p \geq 0.05$  – versus against ischemia/reperfusion group data.

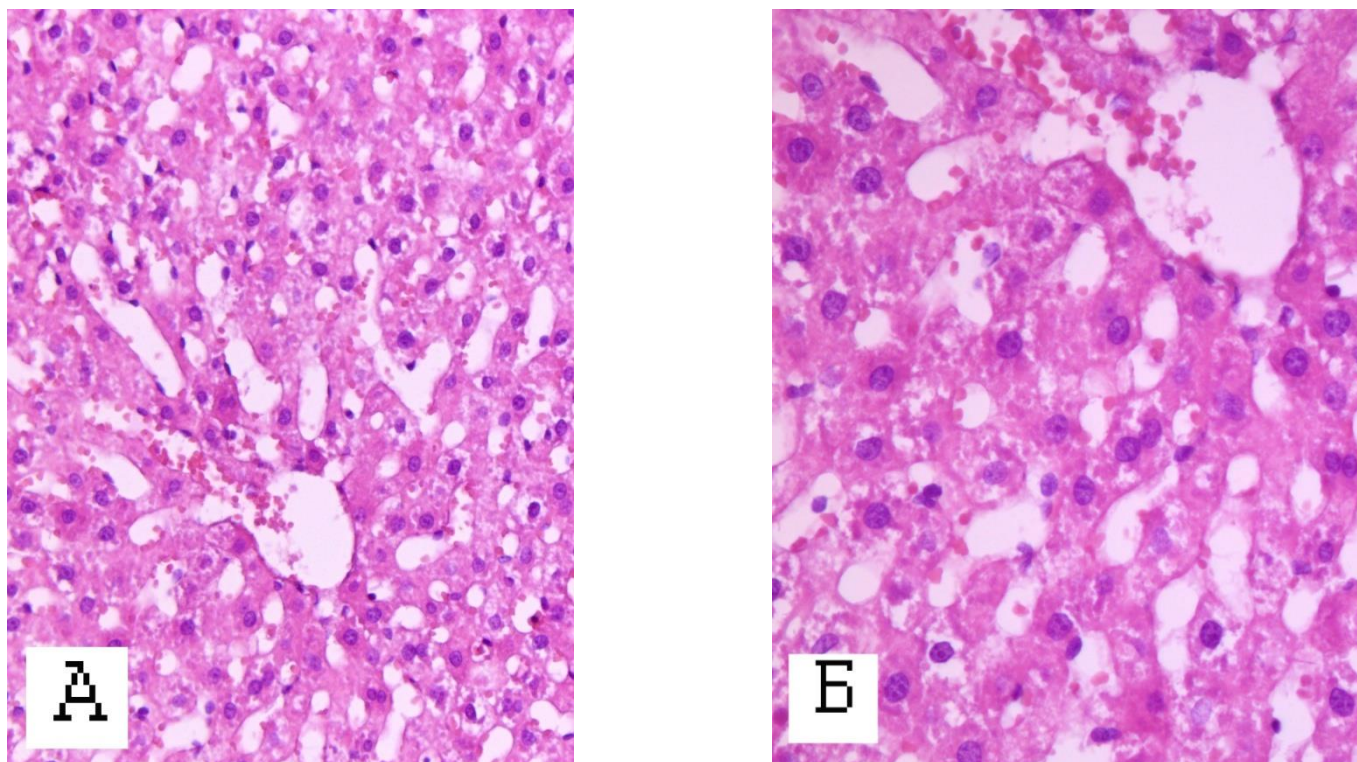
Histological examination showed complex of nonspecific changes caused by ischemic damage and characterized by portal vessels and sinusoids desolation combined with pronounced dystrophic, necrobiotic hepatocytes changes and microcirculation impairment (Fig. 1.). Reperfusion injury appear as severe sinusoidal dilation with diapedetic bleeding increasing dystrophic and necrobiotic changes (Fig. 2.).

Human recombinant erythropoietin injection (Epocrin) 50 IU/kg decreased hepatocellular damage and manifested in necrobiotic changes absence in late stages of 30 minute of ischemia and their small presence at 30 minute of reperfusion. It's characteristic in group with EPO to haven't microthrombosis and stromal leakage (Fig. 3, Fig. 4.).

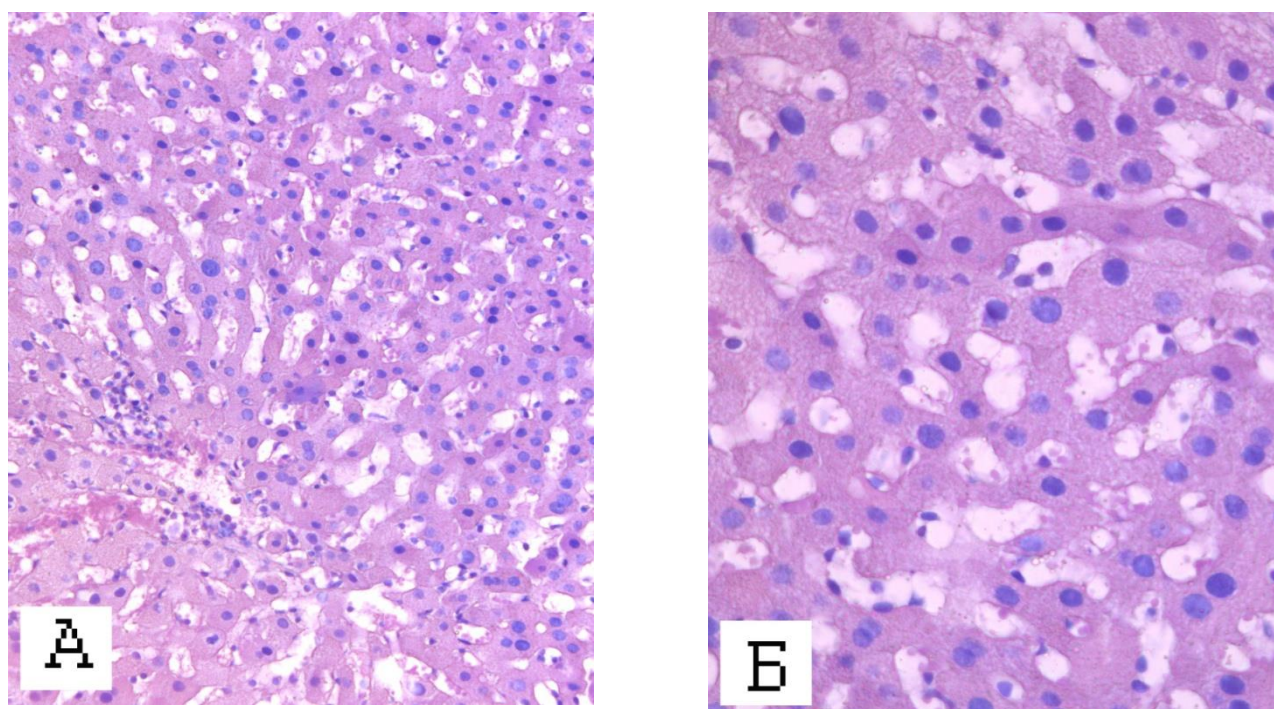


**Figure 1.** Ischemic liver injury: centrilobular anaemia, compact grain liver dystrophy. Hematoxylin and eosin dye. Microphoto. A) X 200. B) X400



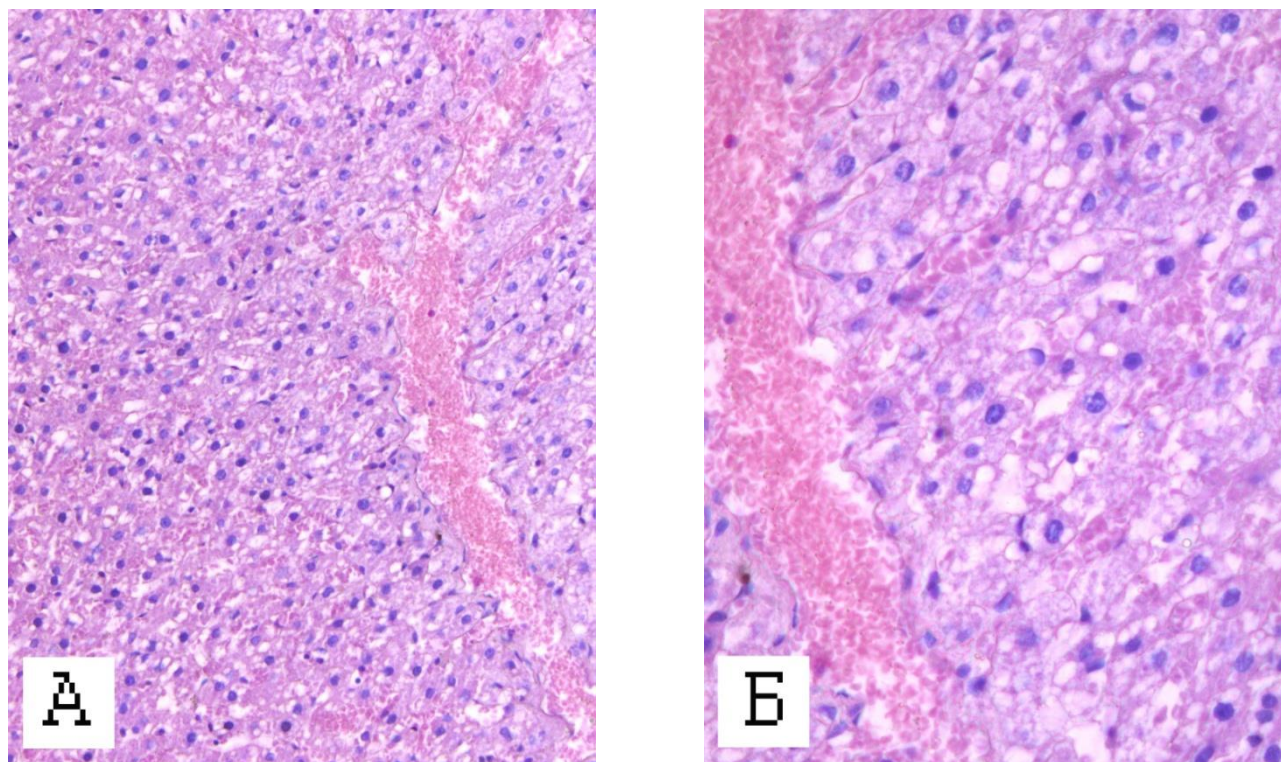


**Figure 2.** Reperfusion liver injury: severe necrotic and dystrophic changes, diapedetic haemorrhage focus. Hematoxylin and eosin dye. Microphoto. A) X 200. Б) X400



**Figure 3.** 50 IU/kg erythropoietin influence on ischemic liver injury: mild centrilobular anemia and absence of dystrophic changes. Hematoxylin and eosin dye. Microphoto. A) X 200. Б) X400





**Figure 4.** IU/kg erythropoietin influence on reperfusion liver injury: venous hyperaemia, absence of hemorrhage. Hematoxylin and eosin dye. Microphoto. A) X 200. Б) X400.

**Conclusion.** Thereby the study found that the recombinant erythropoietin dose-dependently prevented the development of reactive hyperemia 15 minute reperfusion. Prekonditsionuyushey optimal dose is 50 IU/kg. The positive effect of recombinant erythropoietin also confirmed by morphological study and is manifested in the absence of thrombosis and hemorrhage, minimum changes in the severity of necrobiotic a tsentrolobulyarnyh necrosis and venous plethora

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